A Novel 4-cM Minimally Deleted Region on Chromosome 11p15.1 Associated with High Grade Nonmucinous Epithelial Ovarian Carcinomas

Karen H. Lu, Jeffrey N. Weitzel, Srilatha Kodali, William R. Welch, Ross S. Berkowitz, and Samuel C. Mok

Laboratory of Gynecologic Oncology, Division of Gynecologic Oncology, Department of Obstetrics, Gynecology, and Reproductive Biology [K. H. L., S. K., R. S. B., S. C. M.], and Department of Pathology [W. R. W.], Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, and Clinical Cancer Genetics, City of Hope National Medical Center, Duarte, California 91010 [J. N. W.]

Abstract

Prior cytogenetic and restriction fragment length polymorphism studies have demonstrated that allelic deletion of chromosome 11p is common in human invasive epithelial ovarian tumors. To construct a highly detailed deletion map of chromosome 11p, we used 13 polymorphic microsatellite CA repeat primers to identify regions harboring potential tumor suppressor genes. Twenty-three of 48 samples (48%) of invasive epithelial ovarian cancer showed LOH involving at least one locus, consistent with prior studies. None of the five mucinous tumors showed allelic deletion at any of the 13 primers, suggesting that loss of heterozygosity at chromosome 11p may not be involved in the pathogenesis of mucinous ovarian cancer. Two separate minimally deleted regions were identified in nonmucinous ovarian cancer. The first is an 11-cM region on chromosome 11p15.5—15.3 that extends from D11S2077 to D11S988 and includes the HRAS locus. The second is a novel 4-cM region on 11p15.1, defined by marker D11S1310. Deletion of both regions at 11p15.5—15.3 and 11p15.1 is strongly associated with high grade nonmucinous epithelial ovarian cancer.

Introduction

Epithelial ovarian cancer is the fourth most common cancer in American women, and it is the leading cause of death among gynecological malignancies. Little is known about the molecular pathogenesis of this disease. Most likely the progression from benign ovarian surface epithelium to invasive carcinoma is the result of an accumulation of genetic alterations involving oncogenes, tumor suppressor genes, and DNA mismatch repair genes. Although no specific tumor suppressor genes for invasive ovarian epithelial cancer have been cloned, LOH studies have implicated a number of chromosomal sites as regions harboring potential tumor suppressor genes. Frequent LOH has been observed on chromosomes 3p, 6q, 7q, 8q, 9p, 11, 12, 13, 16, 17, 19p, and Xq (1—6). Chromosome 11p was initially implicated as a region with potential tumor suppressor genes from cytogenetic data showing deletions of 11p in the karyotypes of ovarian cancer tissue (7). Using Southern blot analysis, early LOH studies showed 30—50% allelic deletion of the HRAS gene on 11p15.5 (8, 9). However, subsequent studies showed no specific mutations of the HRAS gene, suggesting that a tumor suppressor gene may instead be in close proximity to HRAS (10, 11). Vial et al. (8) and Vandamme et al. (9) used Southern blot analysis to identify the extent of the region of loss surrounding HRAS, as well as other areas of allelic deletion on chromosome 11p. The regions of chromosomal loss found by these authors are too large to attempt cloning of potential tumor suppressor genes. The recent discovery of polymorphic microsatellite CA repeat primers has allowed more detailed mapping of chromosomal regions. We have constructed a highly detailed deletion map of chromosome 11p, and we present two minimally deleted regions on chromosome 11p that may harbor tumor suppressor genes important in the development of high grade nonmucinous epithelial ovarian cancers.

Materials and Methods

Specimen Collection and DNA Extraction. Surgical specimens of human invasive ovarian cancer tissue were obtained from 48 patients under a protocol approved by the Human Subjects Committee of the Brigham and Women's Hospital. The corresponding control tissues consisted of segments of normal fallopian tube, uninvolved round ligament, or peripheral blood. All histopathological diagnoses and grades of the ovarian cancers were confirmed by a gynecological pathologist. All cases were surgically staged according to FIGO criteria (International Federation of Gynecology and Obstetrics, 1987). The invasive tumors consisted of 35 serous, 5 mucinous, 4 endometrioid, 3 mixed epithelial, and 1 undifferentiated carcinoma of the ovary. DNA extraction was performed using methods published previously (6).

PCR and LOH Studies. Using polymorphic CA repeat microsatellites located at 13 loci along chromosome 11p (Research Genetics, Huntsville, AL), PCR amplification was performed on tumor and corresponding normal samples. The map positions of the primers are shown in Fig. 1. The forward primer of each target was end-labeled with [γ-32P]ATP using T4 polynucleotide kinase (Boehringer Mannheim, Indiana, IN). The reaction mix was then diluted into a final volume of 320 µl of primer-PCR mixture containing 40 µl of 10× PCR buffer (0.1 M Tris-HCl, 0.5 M KCl, pH 8.3), 2—5 mM MgCl2, 20 µl of 2.5 mM deoxynucleotide triphosphate mixture, and 2 µl (10 units) of Taq polymerase (Perkin-Elmer, Norwalk, CT).

PCR amplification was carried out with 50 ng of genomic DNA. Conditions for each primer set for denaturation, annealing, and elongation were obtained from the Human Genome Base. Five µl of the PCR product was added to 45 µl of loading buffer containing 95% formamide, 20 mM EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol FF (Sigma Chemical Co., St. Louis, MO), loaded onto a 6% polyacrylamide gel, and run at 1700 V. The gel was then dried and exposed to X-ray film with an intensifying screen. The autoradiographs were then developed and analyzed to determine if LOH had occurred. Cases were considered informative when heterozygosity was detected in the normal control tissue. LOH was defined as the visible reduction of 50% or more in the band intensity of one of the tumor sample alleles as compared to the normal sample.

Statistical Analysis. A χ2 test was performed to compare LOH frequencies between histopathological types, stages, and grades of the cases. Statistical significance was considered when P values <0.05.

Results

Forty-eight invasive ovarian epithelial cancers were studied using 13 primers located along chromosome 11p. The map position and
1cM
2cM
1cM
2cM
5cM
1cM

DELETION MAPPING OF CHROMOSOME 11p IN OVARIAN CANCER

Fig. 1. Summary of the 13 loci on chromosome 11p studied, showing map position and physical distance (12–14).

genetic distance between primers are based on the Genethon chromosome 11 map and CEPH consortium linkage map (12–14) and are shown in Fig. 1. Twenty-three of 48 ovarian cancer tissues (48%) showed LOH involving at least one locus on chromosome 11p. The frequency of LOH at each locus is summarized in Table 1 and ranges from 8% at D11S2071 to 41% at TH. The entire region from 11p15.5–15.2, defined by the primers D11S2071, HRAS, D11S1363, D11S922, TH, D11S1318, D11S1333, and D11S988, shows a consistent LOH rate from 31–41%. The LOH rate falls to 8% at primer D11S2071. A second peak occurs at primer D11S1310 in 11p15.1 with a LOH frequency of 32%.

The deletion map of the 23 samples showing loss on chromosome 11p is shown in Fig. 2. Of the 23 samples, three showed LOH at all informative markers studied, suggesting loss of the entire arm. Analysis of the remaining samples suggests that two separate minimally deleted regions on chromosome 11p in invasive epithelial ovarian tumors can be defined, one larger region in 11p15.5–15.3 and a smaller region in 11p15.1. The larger region extends from marker D11S2071 to D11S988 and is 11 cM in size. The smaller 4-cM region is defined by marker D11S1310 and is flanked by D11S926 and D11S899. Fig. 3 shows autoradiographs of representative cases (443, 332, and 527) that are critical in helping to define the smallest common area of loss in both the 15.5–15.3 and 15.1 regions. Case 443 in particular contains both smallest regions of loss.

Table 1 shows the frequency of LOH by clinicopathological characteristics. The LOH percentage of the serous tumors is higher than the percentage of all types combined for each of the primers studied. Interestingly, none of the five mucinous tumors showed evidence of LOH at any of the 13 primers along chromosome 11p, suggesting that allelic deletion of chromosome 11p may not be involved in the pathogenesis of mucinous tumors. Poorly differentiated tumors showed a higher percentage LOH at all primers in the 15.5–15.3 region and the 15.1 region. No significant correlation between stage and LOH rate was found.

Ten of the 23 tumors showing loss on chromosome 11p (43%) show loss only of region 11p15.5–15.3. These include tumors of all...
grades. Twelve of 23 tumors (52%) show loss of both regions 11p15.5–15.3 and 11p15.1, and 10 of 12 of these tumors were high grade or poorly differentiated.

Discussion

The results presented here support prior studies demonstrating that allelic deletion of chromosome 11p is common in invasive ovarian cancer. Earlier LOH studies using Southern blot analysis focused on HRAS on chromosome 11p15.5. A consistent HRAS allelic deletion rate of 30–50% was seen in all of these studies and is similar to our rate of 35% (1–3, 8, 9, 15–17). However, subsequent studies have shown no specific mutations on HRAS, suggesting that a tumor suppressor gene may instead be in close proximity to HRAS (10, 11). Viel et al. (8) and Vandamme et al. (9) used Southern blot analysis to look for allelic deletion proximal to HRAS and found extensive regions of LOH at 11p15.5 and 11p13. The recent discovery of polymorphic microsatellite CA repeat primers has allowed us to create a more detailed deletion map of chromosome 11p, which may help in the identification of tumor suppressor genes on this chromosome arm.

We have found two separate minimally deleted regions on chromosome 11p, as illustrated in the deletion map. The larger region includes HRAS and extends from D11S2071 to D11S988. The frequency of LOH in this region ranges from 31% at primer D11S2071 to 41% at primer TH. This region maps to 11p15.5–15.3 and is approximately 11 cM in length. We have used eight, closely spaced primers in this region. Because the majority of cases show a continuation of losses at multiple sites, we believe that this region represents a single, large area of loss from 11p15.5–15.3. Gabra et al. (17) used four primers along 11p and found a similar large region of loss at 11p15.5–15.3.

Our second region of loss in 11p15.1 has never been reported previously. This 4-cM region is defined by marker D11S1310 and flanked by markers D11S926 and D11S899. The frequency of LOH for this region (32%) is slightly lower than the frequency seen in the larger, more distal region. Loss of this region occurs in combination with loss of the more distal region. The deletion map generated from cases 332, 443, and 527 illustrates this point. Twelve of the 23 tumors showing LOH along chromosome 11p (39%) show loss of both regions.

At all loci in our minimally deleted region in 11p15.5–15.3, the LOH percentage for poorly differentiated tumors was higher than for moderate and well-differentiated tumors. This finding is consistent with Kiechle-Schwarz et al. (18) and Zheng et al. (19), who both reported that loss of 11p15.5 correlated with poorly differentiated tumors. In our second minimally deleted region in 11p15.1, 10 of the 12 cases showing loss were poorly differentiated. The remaining two cases were a well-differentiated and moderately differentiated tumor.

Although the association of loss of this region to tumor grade was not statistically significant (P = 0.2), our numbers of well- and moderately differentiated ovarian cancers were small.

Twenty-one of the 23 tumors showing LOH on chromosome 11p
were serous adenocarcinomas. None of the five mucinous tumors in our study showed LOH at any of the 13 markers used. Because the mucinous histological type accounts for less than 10% of ovarian carcinomas, we were only able to include five mucinous samples in our study. In Vandamme’s study, there were two mucinous tumors, neither of which showed LOH at any of the four primers used (9), and in Kiechle-Schwarz’ study, none of the five mucinous tumors showed LOH at any of four primers along 11p (18). We hypothesize that deletion of a tumor suppressor gene(s) on 11p may not be involved in the pathogenesis of mucinous ovarian carcinomas. Our laboratory and others have shown previously that K-ras mutations occur at statistically significant higher rates in mucinous than in serous tumors, providing additional evidence of the distinct molecular etiologies of these histological types of epithelial ovarian cancer (20, 21).

Deletions of chromosome 11p can also be found in breast, non-small cell lung, bladder, and testicular cancers (22—25). Recent detailed deletion mapping of 11p in breast and non-small cell lung cancer shows overlap of these regions with our 11p15.5—15.3 region (22, 23). Inactivation of the same tumor suppressor genes located in this region may play a role in the pathogenesis of a heterogeneous group of human cancers. This would be consistent with other more well-characterized tumor suppressor genes such as p53.

**HRAS** on 11p15.5 has previously been shown not to be involved in ovarian cancer (9—11). Specific genes in the 11p15.5 region not yet tested for mutations in ovarian cancer include the insulin-like growth factor 2 and the cyclin-dependent kinase inhibitor 1C. No specific genes have been mapped to the 11p15.1 minimally deleted region found in our study. However, the small size of the region may allow for direct cloning and identification of a potential tumor suppressor gene.

In conclusion, we report the identification of a 11-cM and a novel 4-cM minimally deleted region on chromosome 11p in invasive ovarian cancer associated with poorly differentiated, nonmucinous tumors.

**References**


A Novel 4-cM Minimally Deleted Region on Chromosome 11p15.1 Associated with High Grade Nonmucinous Epithelial Ovarian Carcinomas


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/57/3/387

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.